PB-216 912

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1 July 1956

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A REVIEW OF LITERATURE AND UNPUBLISHED RESEARCH

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U. S. Department of Health, Education, and Welfare
Public Health Service
July 1, 1956

PUBLIC HEALTH SIGNIFICANCE OF PARALYTIC SHELLFISH POISON*

The prevention of poisoning due to the ingestion of toxic shellfish has been a problem of mutual concern to public health and fishery authorities in Canada and United States for many years and has been recognized for over a century as a clinical entity. From the standpoint of public health, paralytic shellfish poisoning cannot be classified as a major problem; however, it has caused considerable concern because of its extreme toxicity and the fact that there is no known antidote. Less than one millionth of a gram is sufficient to kill a mouse, and the fatal dose for man is only a few milligrams.

During the 1945-46 clam canning season, which was cut short by the regulatory action of the Food and Drug Administration, Southeastern Alaska operators produced packs of frozen and canned butter clams valued at over $170,000. As this industry was a winter operation, offering employment and income during an otherwise slack season, it was of special importance to resident Alaskan economy. Likewise, the Maritime Provinces of eastern Canada are an important producing area for soft shell clams, normally exporting about four million pounds of clams per year to the United States.

*Presented by Dr. Keith H. Lewis at the annual meeting of the National Shellfisheries Association and the Oyster Institute of North America, Miami Beach, Florida, July 30-August 2, 1956, and subsequently included in the proceedings of the meeting.

Because of these commercial operations and the popular summer sport of beach parties or "clam bakes", it is obvious that unless adequate control measures are maintained mass intoxication could result.

Of the many naturally occurring poisons, there are only two general types which resemble shellfish poison in some respects. One of these is the so called "waterbloom poisoning"(41) which may be elaborated by the profuse growth of fresh water algae or by their subsequent decay. Farm animals have died within an hour after drinking from lakes where the plankton grows, and it has been possible to demonstrate the poison in solution in the water. Deer and ducks and other wild birds have also reportedly been killed by this poison in various parts of the world. There are no reported cases of humans being poisoned by drinking water containing large quantities of algae. However, during the great droughts of the 1930's there was a widespread unexplained outbreak of gastroenteritis in several eastern cities. Some investigators thought that this outbreak might have been related to the tremendous growths of algae which occurred in rivers used as a source of raw water, even though the water was purified in efficient water treatment plants and was heavily chlorinated.

The other related group of poisons are found in the flesh and organs of fish. Three different types of fish poisoning have been intensively investigated by Halstead(42), but little is known concerning the origin or nature of these poisons. One of the types, Ciguatera, is common in the Carribean and in several instances has been reported as causing intoxications in Florida. The great barracuda is one of

the several fish species involved, and is the most frequently associated with poisoning in Florida. A second type of fish poisoning, the Pacific, is found throughout the South Pacific areas and is generally associated with the coral belt. The third type of poison, Tetrodon, is found primarily in the Japanese areas. Of the three types, the Tetrodon is the most deadly and causes symptoms[3] most similar to those of shellfish poisoning in man and animals.

SOURCE OF THE POISON

The original source of the poison in shellfish is found in certain species of unicellular microscopic marine organisms of which the dinoflagellate, Gonyaulax catenella is perhaps the best known(1). It is a free-swimming organism, multiplying by formation of chains of two, four, or even eight individuals of dark orange or greenish brown color, and living like a true plant cell by photosynthesis. Like all plankton organisms it is most abundant in the summer. At times it may multiply to as large a number as 40 million per liter. At such times the water for miles presents a deep rust color-- the so-called "red water" in the day time and a beautiful luminescent spectacle at night. Needless to say, other dinoflagellates or diatoms may reach similar population densities in the ocean without being poisonous. Apparently, Gonyaulax catenella may vary considerably in number and perhaps, in its poison content, because even small numbers which are not visible as red water may sometimes cause shellfish to become poisonous.

The plankton serves as a food for many animals of the seashore, and various types of plankton feeders may at times become poisonous. There is only one known case in which oysters(44) might have been toxic. Scallops may become highly toxic, but they are of limited public health significance because ordinarily only the non-toxic adductor muscle(9) is eaten. The principal types of shellfish which reach dangerous levels of toxicity and may be consumed by man are mussels and clams. After the mollusks ingest the plankton, the poison tends to accumulate in the digestive gland of mussels(2) and clams, although the gills of the soft shell clam(9) are a secondary site of accumulation and the siphons of

butter clams[8] have been found to contain over 50 percent of the poison. The poison evidently does not harm the shellfish. The resulting toxicity is proportional to the number of Gonyaulax ingested and to their poison content. If a large number of Gonyaulax is present in the water, the toxicity of the bivalves may rise to dangerous levels within a few days. In the absence of the organisms, the stored poison is slowly eliminated.

The reasons advanced by Sommer et al.[2] for believing the dinoflagellate, Gonyaulax catenella, to be the primary source of the poison on the west coast of the United States can be enumerated as follows: (1) In the stomach of toxic mussels this type of plankton organism has been found in considerable numbers, whereas it was observed either not at all or only in very small numbers in the stomach of nontoxic mussels. (2) The results of years of investigation by qualitative and quantitative methods indicate that the yearly maxima of certain species of the genus Gonyaulax occurs preceding and during each poison period. There are other plankton species such as the diatoms which may appear in numbers that greatly exceed those of the genus Gonyaulax, but these increases are not accompanied by an increase in the toxicity of the mussels. (3) When poisonous mussels were kept in the laboratory in clean aerated sea water without food, the toxicity of the mussels dropped about one-half in ten days. On the other hand, when fresh sea water was fed to mussels in the laboratory at a time when Gonyaulax catenella was abundant, the poison content rose as much as twenty times. (4) When surface water to the amount of 150 liters was filtered through a No. 25 net and the residue collected in a volume of 72 ml., a count of the organisms revealed a total of

2,100,000 Gonyaulax catenella, and after extraction of these organisms with acid, it was revealed that 2,050 of these organisms yielded 1 lethal dose (mouse). Riegel, et al.(31), also describe an experiment in which about 500,000 mouse units of poison were centrifuged from 5,000 liters of sea water taken from a red water area containing Gonyaulax.

In studying the paralytic poisoning associated with mollusks from the Bay of Fundy, Needler(4) concluded that the toxicity was caused by Gonyaulax tamarensis. Koch(5) found that another dinoflagellate, described as Pyrodinum phoneus was responsible for extreme toxicity in Belgian mussels. In contrast to mussels from California and New Brunswick, the toxic Belgian mussels originated from estuaries and inner harbors. In this case it must be assumed that an organism is responsible which has its habitat in brackish waters. Further taxonomic studies are needed to establish the relationship of these species to Gonyaulax catenella.

In an attempt to correlate shellfish toxicity with other periodic manifestations, one naturally turns to the meteorological and oceanographic data. From a study of the weather records in California it is apparent that the maxima of shellfish toxicities occur in the summer at times when the water and air are relatively cool(3). The temperatures of the water along the coasts of California and Oregon indicate that there are upwellings of colder water to the surface. Consequently the average temperatures of the water along the central coast of California are 3° to 4° lower than the average to be expected from the latitude of the locality. In fact, the average temperatures of the water along the entire region in which mussel poisoning occurs, from

central California to Alaska, are remarkably similar in summer time, ranging from 10° to 14° C.

In regard to the tides[3], no clear relationship can be observed. As a general rule the toxicity may be expected to reach a maximum on or immediately after the second big tide in early summer, but outbreaks have occurred at the time of the autumnal equinox when tide differences were at a minimum. Evidently tides are not a primary cause of variations in the toxicity of shellfish.

In studying the effect of water conditions on Gonyaulax found in the Bay of Fundy, records were kept showing the state of the tide, time of day, condition of the weather, and surface and botton temperature of the water. Of these, the only factor correlated with the occurrence of Gonyaulax tamarensis was the temperature of the surface water. Comparison of the numbers of Gonyaulax tamarensis and water temperatures, especially at the surface, indicate that Gonyaulax tamarensis may appear any time after the surface water reaches 10°C. Small peaks in the counts of Gonyaulax tamarensis often occur at the same time as temperature peaks during late July or early August, but the highest count of Gonyaulax tamarensis for any given year corresponds to the peak temperature (13.9°C) in the latter half of August. Although these findings have provided a useful basis for local control measures, the specific relationships between water temperature and toxic plankton are not characteristic of the St. Lawrence River estuary and perhaps other areas.

In the Bay of Fundy the principal enemy of Gonyaulax has been found to be a ciliate[4], Favella ehrenbergii. The ciliate occurs at about the same time of year as Gonyaulax, sometimes in enormous

numbers. It was found to be feeding on small dinoflagellates, including Gonyaulax tamarensis, and investigation showed a relationship between the counts of the two species. Large numbers of Gonyaulax tamarensis occurred only when Favella ehrenbergii was rare or absent. A rough survey of the diatom production in this area also indicated that the number of diatoms(4) may have some effect on the number of Gonyaulax tamarensis. It is suggested that when many diatoms are present in late July or August they may compete with the Gonyaulax for food or reduce the light and so check production.

During the summer of 1949 the Hooper Research Foundation of the University of California cooperating with the Fishery Products Laboratory in Ketchikan, Alaska, successfully identified Gonyaulax catenella in waters from several areas of Southeastern Alaska(7). The organism was never found in numbers even approaching the concentration needed to cause a visible red tide. Red tides do occur during the summer, but they have been found to contain concentrates of other plankton which do not exhibit the toxic qualities of Gonyaulax. However, in Alaska shellfish are found to be toxic the year around(8) with a slight indication of a period of high toxicity in the fall followed by a decrease during the winter and a rise again in the spring. It is, therefore, probable that the water temperature does not fluctuate so greatly in Alaska, and that there are Gonyaulax present most of the time although the temperature never becomes high enough for blooms of these organisms to occur.

OCCURRENCE AND DISTRIBUTION OF TOXIC SHELLFISH

The first recorded death ascribed to paralytic shellfish poison on the North American Continent occurred on June 15, 1793, on Vancouver Island, British Columbia, when one of Captain Vancouver's seamen died after eating roasted mussels. There is reason to believe that this was not the first death due to paralytic shellfish poison for Vancouver wrote that his crew members had some idea of how to treat the stricken seamen, thus indicating prior knowledge.

In 1799[43] a troup of Aleut hunters from Unalaska and Kodiak stopping at a place now known as Peril Way (near Sitka, Alaska), consumed mussels and according to Petroff 100 men died in less than two hours.

In 1953 Meyer[10] reported that the Hooper Foundation had collected histories of more than 400 cases of mussel or clam poisoning recorded in the literature[3, 15,45,46]. There were 35 deaths among the group. Most of these poisonings occurred at irregular intervals along the central California coast with a sprinkling of extensive outbreaks from Juneau, Alaska, to southern California. All cases occurred between May 15 and October 26.

Medcof[9] and his associates and Needler[4] reported a total of 28 cases of paralytic poisoning due to the soft shell clam, Mya arenaria, in the areas of the Bay of Fundy, New Brunswick, and Nova Scotia in 1945. In 1936 Murphy[11] reported five cases with two deaths caused by eating mussels in Nova Scotia. Twenty-four cases[47] were reported in Maine in 1943.

Several cases attributed to the ingestion of the white mussel (Donax serra) or the black mussel (Mytilis edulis) have been observed

near Cape Town, South Africa[12-13]. There were 12 cases with 4 deaths due to these shellfish in Belgium[14].

Mussel poisoning has also occurred in New Zealand[10], and before 1915 Germany, England, Ireland, and France had reported over a period of twenty years approximately 110 cases with 24 deaths[15].

In 1948 two children died from eating mussels collected at Les Boules, Quebec, on the south shore of the St. Lawrence River, and in July 1954 two out of seven persons died from eating soft shell clams collected at Metis Beach[16].

One fatal case of shellfish poisoning was also reported in July 1954 at False Pass, Alaska[17]. Six others eating mussels from the same source four days earlier became sick. A summary of known outbreaks by geographical location, year of occurrence, and number of cases is given in Table I. The disastrous outbreak in Alaska in 1799 has been omitted from the table because the description of the chemical manifestations is not sufficiently detailed to establish conclusively that mussel poison was the cause of illness.

In the above cases of shellfish poisoning it has been assumed that fresh mussels and mussels kept for a few days were equally toxic. In California[3] all of the mussels were derived from the open shore of the ocean and were of the species Mytilus californianus. Not a single poisoning occurred from eating mussels of the species Mytilus edulis, gathered in San Francisco Bay or other bays. On the west coast nearly all clam poisonings were caused by Saxidomus nuttallii[3] (Washington clam) from Bodega Bay. However, Siliqua patula (razor clams) have, on several occasions, been found to contain only a little less poison than mussels from a nearby source. The The toxicity curve of sand crabs has also been found to parallel that of mussels.

TABLE I

Known Cases of Paralytic Shellfish Poisoning

1798 to 1954

Location	Years of occurrence	No. cases	No. deaths
California	1903, '15, '17, '18, '27 '29, '32, '36, '39, '43 '44, '46, '48, '54	373	30
Oregon	? , 1933	> 22	1
Vancouver, B.C.	1793	3	1
Alaska	1934, '54	19	3
Nova Scotia & New Brunswick	1936, '45	33	2
Maine	1948, '54	> 24	-
Quebec	1948, '54	9	4
England	1857, '72, '88, 1904	> 7	4
Wales	1909	19	1
Scotland	1827	30	2
Ireland	1872, '90	11	8
Norway	1901	5	2
Prussia	1885, '87	22	5
France	1907	13	2
Belgium	1940	12	4
South Africa	1948	several	-
New Zealand	1951	several	-
	TOTAL	> 602	69

The data accumulated indicate that along the Pacific coast[3] of the United States the time of danger from shellfish poisoning is from May until October with peak toxicity about the middle of July. The variation in toxicity in mussels in a given bed is not large, i.e., not more than ± 50 percent, and is negligible compared with the variability in samples from various beds which amounts to as much as 1:3500. Several assays were also made of male and female mussels and toxicity was always found the same within the limits of experimental error. These constancies held, however, only if mussels from approximately the same tide level were compared. Specimens gathered from the lowest possible locations, which are swept by the waves most of the time, have on the whole been found more poisonous than those from the upper limits of their habitat, where the water supply may be scarce.

In studying the presence of poison in the butter clam, Saxidomus giganteus, in Alaska, it was concluded that the clams were toxic during every month in certain areas, although there was a slight indication of a high toxicity period in the fall, followed by a decrease during the winter and a rise again in the spring. In most cases there was no significant difference in samples taken at the same time from high and low plots[8]. It was further shown that the bodies of the clams do not vary greatly in toxicity from month to month but the toxicity of the siphons showed a marked fluctuation.

In Alaska none of the beaches[6] from so-called outside waters contained toxic clams, but the beaches on or near the open water of the wide straits characteristic of southeastern Alaska yielded the most toxic clams. As sampling was continued toward the head of the bays the toxicity decreased.

Poisonous shellfish gradually lose their toxicity if they are put into sea water[3] in nontoxic surroundings. With strongly poisonous shellfish, the excretion of the substance into the water can be demonstrated directly. Experimental work in California showed a drop to one-half of the original toxicity in ten days when mussels were kept in filtered aerated sea water at room temperature. However, the Alaskan butter clam[7] and the east coast soft shell clam[9] lost their toxicity much more slowly in transplanting studies. On the other hand, by transplanting nontoxic shellfish to toxic areas, the shellfish become poisonous in three to four days[3]; in fact, some poison was detected after 24 hours. In California, in one area, mussels showed a hundred-fold increase in toxicity in about twelve days. At Metis Beach, Quebec[16], on the south shore of the St. Lawrence River it was shown in 1954 that within six days the toxicity of Mya arenaria increased from 542 mouse units to 26,180 mouse units per 100 grams. These facts indicate that slightly poisonous shellfish may become dangerous in the course of a few days and demonstrate the importance of the problem from the standpoint of public health.

PHYSIOLOGY AND TOXICOLOGY

The symptoms of poisoning[15] in human beings are primarily peripheral paralysis which may vary from a slight tingling and numbness about the lips to a complete loss of strength in the muscles of the extremities and neck, and to death by respiratory failure. In a moderately severe case the tingling, stinging sensation around the lips, gums and tongue develops about five to thirty minutes after the consumption of the mussels. This is regularly followed by a numbness or a prickly feeling in the finger tips and toes, and within four to six hours, the same sensation may progress to the arms, legs and neck, so that voluntary movements, as for example raising of the head, can be made only with great difficulty. In all cases of moderate severity this toxic weakness and stiffness of locomotion is accompanied by a peculiar feeling of lightness. Some patients have declared that they experienced a floating or flying sensation and even heavy objects appeared to them very light. The reflexes are normal and active. It is stated that in one of the fatal cases the deep reflexes were affected. The mentality remains clear, although dizziness, staggering, and drowsiness may be noted. A few patients complain of a gripping sensation around the throat and slight respiratory distress. Incoherence of speech was noted in one of the fatal cases. Vomiting is an inconstant symptom, but diarrhea and abdominal pain have not been recorded in untreated cases. In fact, there is a tendency to constipation which persists for several days. Records of carefully observed cases show the average temperature to be slightly subnormal (mean 98° F). The pulse is firm and slightly accelerated (80-100 per min.). During recovery some patients have chilly sensations in their

limbs and may feel slightly stupefied or become easily fatigued for a number of days. The longest recorded interval between eating poisonous shellfish and death is ten hours and the shortest interval is three hours. Intoxications may be mild or fail to develop in those who have consumed the shellfish in conjunction with a heavy meal, have boiled the mollusks with rice and garlic, or have fried them in oil. When the shellfish are taken into an empty stomach the intoxication rate seems to be higher. As soon as intoxication is recognized, emptying of the stomach by an emetic and purging by brisk laxatives has been the usual practice, but in severe cases, these measures cannot be relied upon to prevent adsorption of a fatal dose of poison. As soon as difficult breathing develops artificial respiration should be applied. Clinical observations indicate that, even in severe cases, this procedure may prevent a fatal issue if extended over several hours.

The lethal dose of mussel poison for man was not known until 1946, when an epidemiologic investigation[10] furnished leading information. In most instances the amount of poison eaten by the victims cannot be estimated reliably, but in this episode, involving 2 men and a woman, the exact number of mussels eaten by each was known from the number of empty shells left after the meal. Furthermore, adequate samples of cooked and raw mussels for assay purposes were left over from the beach picnic. It was estimated that one member of the party consumed about 42,000 mouse units; this man died in less than four hours. The woman ingested about 22,000 mouse units, became seriously ill and was put in a respirator for four and a half hours. This supportive measure was probably responsible for her recovery. The

second man, who had eaten mussels containing about 17,000 units, had mild symptoms and recovered. If this is an accurate estimate, the lethal dose for man is probably between 20,000 and 40,000 mouse units.

A more recent epidemiological investigation of an outbreak involving 7 persons at Metis Beach[16], Province of Quebec, gives further information on the amount of poison which may be necessary to cause intoxication. One death resulted from the ingestion of not less than 3,300 or more than 31,300 mouse units. A second fatality resulted from the ingestion of not less than 1,200 or more than 11,700 mouse units, but another person who ate a similar quantity survival. Four other persons survived but had severe symptoms from ingestion of 428 to 3,200 mouse units each.

Medcof et al.[9] also investigated clam poisoning in Canada and concluded that the people involved showed great variation in severity of reactions to approximately equal doses. In general, however, those who ingested the most poison suffered most. Mild poisoning, with symptoms sometimes included numbness of legs and arms, arose from doses of poison estimated at 2,000 to 10,000 mouse units. Those severely poisoned, who suffered prostration and paralysis in addition to mild reactions, generally ingested 10,000 to 20,000 units. Respiratory difficulty resulted from the ingestion of heavier dosages estimated at about 30,000 units. The inquiry further showed that picnickers (city people) eat clams only on rare occasions. In contrast, people living in many of the shore communities eat them frequently, especially in winter when other types of protein food are often not readily available. The 88 different persons for whom records of consumption of toxic shellfish were obtained included 35 residents and

53 nonresidents of shore communities. One case of poisoning arose in the former and 23 in the latter group. The maximum dosage ingested without producing any symptoms was estimated at 17,000 units, which is sufficient to produce severe illness in the most susceptible persons. From these records[9] it may be deduced that: (1) some people have a natural tolerance to the poison; (2) some people in the shore communities who consume shellfish more or less regularly may acquire a tolerance to doses of poison which would produce severe symptoms in susceptible persons. The investigators were unable to demonstrate the development of tolerance in experiments with guinea pigs and mice. Their research indicated that the poison is not antigenic, and that repeated exposures do not increase resistance.

In connection with establishing the human lethal dose, it is of interest to compare the estimates for man with results obtained using various laboratory animals. In 1945, '46, and '47 several shipments of frozen clams from Alaska to Seattle, Washington, were detained by the Food and Drug Administration[18] because they contained shellfish poison. Toxicity tests were made to determine the LD_{50} for several different animals. The comparative data in Table II were obtained by using material from a commercially frozen pack containing from 800 to 2,000 mouse units per 100 grams. From 40 to 80 animals of each species were used, except for the monkeys and dogs, where smaller numbers were tested.

As indicated in Table II, these workers, using a crude acid extract of clams, found an oral LD_{50} for rats of 1060 mouse units per kilo. After a supply of purified paralytic shellfish poison became available to the Public Health Service, it was thought of interest to

TABLE II

LD_{50} of Shellfish Poison for Various Animals

Animal	Oral LD_{50} per kilogram*(18)	Estimated lethal dose for adult animal*
Mice	2,100	63
Rats	1,060	320
Monkeys	2,000 to 4,000	15,000
Cats	1,400	3,500
Rabbits	1,000	3,500
Dogs	1,000 approximately	11,000
Guinea Pigs	640	260
Pigeons	500	250
Man	500	36,500

* In mouse units.

TABLE III

Variation in Response of Rats to Oral Doses of Paralytic Shellfish Poison

Strain	Weight Grams	No. tested	Conditioning dose*	Oral LD_{50}*
Hamilton	100-150	40	0	1,715
Sprague-Dawley	200-250	80	0	3,270
Sprague-Dawley	200-250	90	1,000	4,718

* In mouse units.

determine the LD_{50} using this solution. In one experiment at the Sanitary Engineering Center, using 40 white albino rats from Hamilton Laboratory Animals, Inc., in Cincinnati, an oral LD_{50} for male and female animals weighing 100 - 150 grams was determined to be 1715 mouse units per kilogram. In another experiment, using 80 adult male and female albino rats of the Sprague-Dawley strain weighing 200 - 250 grams, the oral LD_{50} dose was established as 3270 mouse units[19] per kilogram of body weight. In still another experiment, in which 90 rats of the Sprague-Dawley strain were pretreated with a nonlethal conditioning dose of 1000 mouse units per kilogram 2 weeks prior to determination of the LD_{50} dose for these rats, the LD_{50} dose was 4718 mouse units per kilogram. From these preliminary results summarized in Table III, it can be concluded that the oral LD_{50} dose for rats may vary, depending upon the strain and/or weight of the rats used, and that a nonkilling oral dose of paralytic shellfish poison renders rats less sensitive to subsequent doses. If a comparison can be made between the ID_{50} for rats determined by the Food and Drug Administration and the LD_{50} determined by the Sanitary Engineering Center, it would appear that an LD_{50} dose determined by using purified shellfish poison is higher than when a crude extract of poison is used. This latter result would seem to confirm the finding of an effect of salt[36] in lowering the bioassay of clam meat extracts using mice.

A number of physiological and pharmacological studies have been made on shellfish poison in order to investigate its mode of action. In autopsies on humans[15] no significant findings were recorded in the abdominal or chest cavities except a slight pulmonary

congestion. In every case the folds of the stomach were studded with small hemorrhages.

Injection of small or intermediate amounts of mussel poison into the dog or rabbit[20] causes a definite slowing of respiration with gradual recovery. In the dog there is a fall in blood pressure followed by a secondary rise. With the respiratory depression, a secondary slowing of the heart occurs. Large doses cause an immediate speeding up of the heart followed by a marked secondary slowing. When fatal doses are given the heart continues to beat after respiration has stopped. In one rabbit the heart beat forty-five minutes after respiration had stopped, and when the thorax had been opened, it was found that the auricles were beating 3 to 5 times faster than the ventricles. It is throught that this heart block is probably due to the direct action of the poison on the heart muscle.

In a study of the excretion[20] of the poison, urine was taken from a dog which had been fed 100 mouse units over a period of 2 hours. Of the 100 mouse units, 40 were recovered in the urine, and it was concluded that excretion takes place probably almost as fast as absorption, thereby preventing a high concentration of the poison in the body fluids.

Frogs[20] are quite resistant to shellfish poison. Only a slight transitory stimulation of the respiratory movements is caused by 7.5 mouse units. Fifteen mouse doses are required to cause severe symptoms. The general symptoms resemble closely those caused by curare and consist of progressive paralysis, which is quite general, with the exception of the heart action. The respiration is slowed and may stop, and the heart is slowed, but the animal may recover after

several days depending upon its individual resistance and the environmental conditions.

On macroscopic[21] examination of the central nervous systems of poisoned animals, softening and edema of the brain was observed with moderate congestion of surface vessels. There was no evidence of hemorrhages into the spinal cord or brain. The lungs revealed large and small areas of hemorrhage, and the abdominal viscera were congested with blood. Microscopic[21] examination of the organs revealed the usual changes accompanying agonal death. Acutely poisoned animals revealed no alterations in the nerve cells of the central nervous system. The large nerve cells of the ventral horn of the spinal cord and the ganglion cells of the medulla of the chronically poisoned animal showed alterations in certain cytologic constituents. The only striking evidence within the organs is the altered mitochondria of the convoluted tubles of the kidney. The gradual elimination of the poison by the kidneys may be responsible for this effect.

From a group of experiments on frog muscle and nerves, Kelloway[22] concludes that the shellfish poison is a neurotoxin with central effect upon the cardiovascular and respiration centers and peripheral effect upon the nerve endings, both motor and sensory.

Fingerman et al.[23] however, conclude that shellfish poison has a strong effect on both peripheral nerves and skeletal muscles of the frog. The action of this poison on muscle is similar to that of curare. Furthermore, shellfish poison has an even more general action than curare, for it not only decreases the excitability of muscle to direct stimulation but also blocks nerve conduction. In view of these actions, this poison must be considered as a neuromuscular toxin rather than a neurotoxin.

CHARACTERISTICS OF THE POISON

On the occasion of the Wilhelmshaven mass intoxication in 1885, Salkowski[24] classified the mussel poison as belonging to the group of the chemical poisons of the highest potency. Stability to heat, instability to alkali and solubility in alcohol were established. At the same time, Brieger[25] isolated a gold salt of the base $C_6H_{15}NO_2$ called "mytilotoxin" which he considered a quaternary ammonium base and the pure poisonous principle. According to Muller[27], Thesen[26] in 1902, although obviously dealing with the same type of toxin, could not identify his poison as "mytilotoxin".

Muller[27] in 1948 also attempted to repeat Brieger's method for isolation of "mytilotoxin" but concluded that even in highly concentrated and purified solutions no precipitate of the poison could be formed by either gold or platinum salts. Muller was inclined to believe as a result that the toxic properties of Brieger's mytilotoxin were simulated by traces of poison adherent to an inactive substance. Muller's experiment supports the opinion that "mytilotoxin" does not, as stated in many textbooks, represent the chemically pure poison responsible for the paralytic type of shellfish poison.

Mussel poison has been shown to be soluble in water, methanol, ethanol, glacial acetic acid, and aqueous acetone[15]. The effectiveness of these solvents for extracting the poison appears to be increased if small amounts of hydrochloric acid are added. The poison is insoluble in ether, chloroform, ethyl acetate, butanol, and toluene. It cannot be extracted from an aqueous solution by any of these solvents. The isolation of betaine from a partially purified extract of mussel poison indicated that the properties of betaine and the poison were enough alike

that the substances tended to accompany each other. This fact and the solubility behavior suggested that it might be a nitrogenous base.

Muller(27) further characterized shellfish poison as being of relatively small molecular weight. The purified poison did not give any color reactions for alkaloids and was not removed from solution by the customary precipitants. The poison was purified by the following steps. Drying of the toxic organs, extraction of lipoids with ether, extraction of the poison with acid methyl alcohol, adsorption of impurities and pigments on Norit A, adsorption of poison by permutit, elution by KCl, separation from inorganic salts by the alcohols, acetone, or HCl - ether, and precipitation of poison by rufianic and reinecke acids. The most purified preparation had a potency of 590 mouse units per mg. of solids but still contained 35 per cent ash. Analysis of this purified preparation showed it to contain 10.73 per cent carbon, 5.31 per cent hydrogen and 8.59 per cent nitrogen. Two atoms of nitrogen were present for each three atoms of carbon. The preparation also gave negative carbohydrate and protein tests, was dialyzable, and was not affected by ultraviolet irradiation. Catalytic hydrogenation, as well as attempts at methylation did not change the physiological activity, but acetylation completely inactivated the poison. Hydrolysis in moderate concentrations of mineral acid did not affect the poison. Boiling in strong acid solution destroyed its potency. Oxidants attacked the poison in acid solution only at high temperatures, causing instantaneous destruction at 100°C when treated with H_2O_2. No adsorption of the poison on aluminum hydroxide occurred at various pH values. Kaolin adsorbed about 50 per cent of the poison from an aqueous solution. Brief shaking with

Lloyd's reagent removed the poison quantitatively from aqueous or acid alcohol solutions, but no satisfactory method for elution was found. Common sea sand, washed free of electrolytes, adsorbed approximately 90 per cent of the poison from aqueous solution and practically none from alcoholic solution. Diatomaceous earth did not adsorb the poison or pigments from a crude extract.

Sommer and Bendien[28] investigated the use of chromatography for further purification and found active carbon to be the most satisfactory adsorbent. In preliminary tests to determine the optimum conditions for chromatography, four different carbons were tested, of which Norit A[29] appeared to be the most suitable. The mussel poison was not adsorbed completely from solutions containing less than 1.0 N acid. Traces of ethanol in the extract also prevented complete adsorption of the toxic material. The mussel poison hydrochloride was retained by the carbon, and the major portion of the inorganic, as well as organic, contaminants passed through at once. The use of distilled water to develop the chromatogram was sufficient to carry the poison through into the filtrate. By repeated treatment, as above, the ash content of highly toxic material obtained from carbon columns was reduced to 3 or 4 per cent. By chromatography on carbon of a concentrate obtained by ion exchange on Decalso[30], a mussel poison hydrochloride was obtained with a toxicity greater than 1,000 mouse units per mg. of solids.

Mussel poison extracts, even after preliminary purification, retain varying amounts of other bases. Choline was found in Decalso eluates. After chromatography of the eluates on Norit A, it was found in the primary acid filtrates. Betaine was also found in acid filtrate

from the chromatography of crude extracts on Norit A. A study of the nitrogenous bases[31] was made because their presence in partially purified poison indicated they possessed chemical properties similar to the poison, and because the removal of these bases would be made easier if they were identified. In addition to betaine and choline, homarine, taurine, and tyrosine have been isolated and identified. Choline and homarine, both quaternary bases, remain closely associated with the poison through the preliminary steps in its purification. An additional base has been detected by its characteristic ultraviolet absorption spectrum but has not been identified.

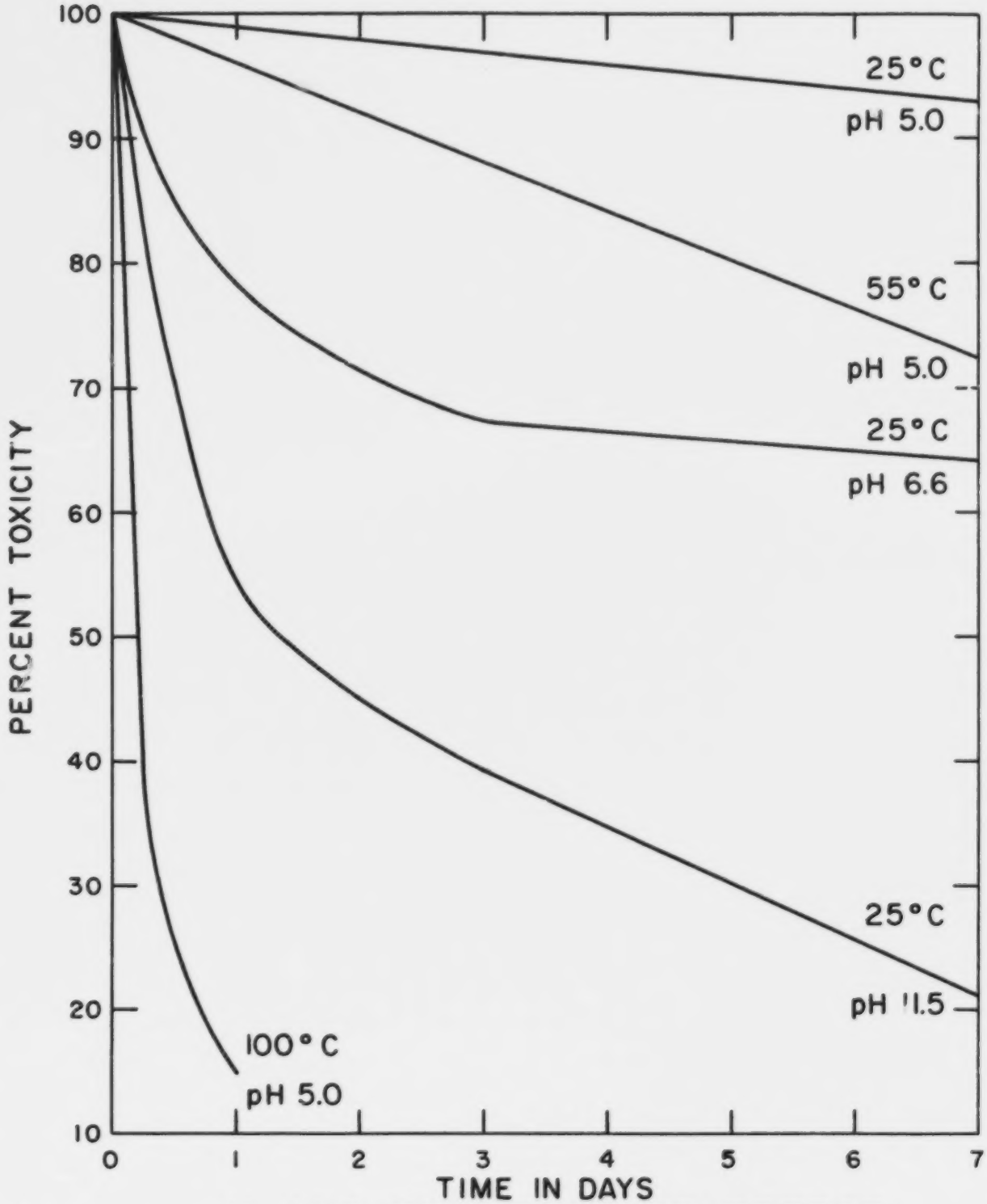
Recently, Schantz, et al.[34,35] have obtained the poisons from California mussels and Alaska butter clams in pure form. These poisons were purified by adsorption on Amberlite IRC-50 followed by chromatography on XE-64 and on acid washed alumina. The potency of the preparations obtained in this way was found to be between 5500 and 6000 mouse units per mg. of solids. The purified poisons were subjected to frontal analysis and counter-current-distribution and were found to be pure. Repeated chromotography on alumina and recrystallization of the poisons as a helianthate did not produce a product of higher toxicity. The chemical and physical properties of the two poisons indicate that they are identical.

Studies[30] were also made of the effect of pH and temperature on the stability of the poison in aqueous solution as measured by its toxicity. The decrease in toxicity with increases in pH and temperature, as illustrated in Figure I, shows that the poison must be handled in acid or, under certain conditions, in neutral solution, and that the

temperature should be kept low. For example, there is a loss in toxicity of only 6 per cent in six days at 25°C, but at a pH of 6.6 this loss increases to 35 per cent, and to 74 per cent at pH 11.5. Likewise an increase in the temperature from 25° to 100° at a pH of 5.0 results in an increase in the loss in toxicity from 6 per cent in six days to 85 per cent in one day. No change in toxicity of shellfish poison is observed in aqueous solution at room temperature if the solution is more acid than pH 4.5, nor in 3 N hydrochloric acid solutions up to a temperature of 55°. These studies, on the other hand, indicate that the commercial processing of clams and mussels by heating above 100°C may be expected to reduce the toxicity considerably unless the product is acidic.

Medcof et al.[9] reported the effects of domestic cooking on toxicity. Their results show that ordinary cooking of shellfish in a little water, even for brief periods, provides the consumer with some degree of protection. On the average, 100 grams of steamed, boiled, or pan-fried meat contained only about 30 per cent of the poison present in 100 grams of raw meat. Cooking may actually reduce the toxicity of the meat by considerably more than 70 per cent because a part of the poison is expressed in the juices. However, when clams are steamed or boiled, varying amounts of the juices may be ingested with the meats, either in the form of bouillon or broth. The temperatures involved in pan frying are considerably higher than in steaming or boiling and seem to be somewhat more effective in destroying the poison even though none of the extractives are discarded.

In 1944, and 1945 a cannery[9] processed 15 different lots of poisonous clams following its established procedure. This involved



pH AND TEMPERATURE EFFECT
SHELLFISH POISON IN AQUEOUS SOLUTION
(SOMMER, et al)

FIGURE 1

27-a

pre-cooking for 15 to 20 minutes in barrels with loose-fitting heads into which live steam was fed. The clams were then shucked, the siphons trimmed off, the meats washed in warm fresh water and packed in cans. Next, a portion of the hot bouillon resulting from the steaming was used to completely fill the cans. The bouillon was seasoned with vinegar altering the pH from about 8.2 to 6.5. The cans were sealed without being exhausted and were retorted at 250°F for 45 minutes. The results showed that under the alkaline condition of steaming and the slightly acid conditions in cans during retorting, 84 to 94 per cent of the poison is destroyed. Presumably canning without adjustment of the pH by addition of vinegar would result in even greater destruction of the poison, since it is less stable in alkaline than in acid media. Further tests showed that the bouillon contains considerable poison. In commercial canning the preliminary cooking with steam produces much more bouillon than is ordinarily used to fill the cans, and the surplus has to be discarded. Thus, much of the poison present in the raw clams never enters the commercially canned product. To illustrate this point, raw shellfish meat was packed in cans without adjustment of pH, sealed and processed by two methods - retorting at 230°F for 55 minutes and in a boiling water bath for 3 hours. The lesser reduction in toxicity (64 to 84%) in these tests indicated that the drainage of bouillon from meats is highly important in producing the low toxicities consistently observed in commercial packs. It was concluded that pre-cooking clams with steam for 10 minutes reduced the poison content by about 90 per cent but extending the period produced no significant additional reduction. Retorting at 250°F for 45 minutes lowered the

toxicity only by an additional 3 per cent.

Although perhaps not true of the soft shell clam, it should be pointed out that with the Alaska butter clam the commercial practice of removing the siphon is highly significant in reducing the toxicity of the canned product, since about one-half to two-thirds of the poison contained in the butter clam is found in the siphon.

Numerous studies on transplanting and on storage in air, on ice, and in the frozen state have been conducted in an effort to find a means of detoxification of raw shellfish. None of these methods have resulted in the development of feasible industrial practices. Studies have also been made of variations in commercial canning and processing, in an effort to find ways to destroy all of the poison. Until more effective methods are found for detoxifying clams and mussels, commercial exploitation of this valuable food supply is not possible in many areas. Recently, preliminary experiments on the destruction of shellfish poison by ionizing radiations have been conducted cooperatively by the Robert A. Taft Sanitary Engineering Center and the Massachusetts Institute of Technology. Through the courtesy of Dr. B. E. Proctor, Head of the Department of Food Technology at M.I.T., we have exposed both toxic clam meat and the purified poison to 3,000,000 rep's. Although the data now available are too limited to be conclusive, the poison appears to be so little affected that irradiation offers no ready means of detoxifying shellfish on a commercial scale.

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PREVENTION AND CONTROL

The prevention of shellfish poisoning involves education and legislation in addition to technical control measures. The plain fact that any type of clam or mussel may be a potential source of poison should be readily understood. Along the Pacific shore of North America and the Canadian Atlantic Coast a safe rule is: do not eat the viscera (dark meat) of mussels or the siphons of clams, or drink the juice of mussels, clams and scallops from the open coast unless the shellfish from that specific area have been tested recently. The white meat must be thoroughly washed before cooking. The addition of baking soda in cooking shellfish has been advocated to help reduce the toxicity, but it is no safeguard against poisoning if highly toxic whole shellfish are prepared, and it may reduce their palatability.

During the poison season of 1939 in California[1] it was shown that warning signs posted along the beaches and across the highways at county lines are effective in reducing the number of persons who dig clams. The same applies to publicity in the local press and through radio stations along the coast. The health officer or local physician has an excellent opportunity to dispel in the minds of the people the many erroneous notions[1] concerning the cause of the shellfish poisoning. Among the points to be emphasized are the following: (1) Paralytic shellfish poison is not a post-mortem product of decomposition. (2) Temporary exposure to the sun does not harm living shellfish, nor does it make them poisonous. (3) Mussels below the tide lines are, if anything, more poisonous than those above water. (4) Copper in the rocks, oil on the beaches, and stagnation or pollution of the water are in no way connected with

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paralytic shellfish poisoning, although they may render the shellfish otherwise unfit as food. (5) Toxic mussels or clams cannot be distinguished from normal shellfish without laboratory tests. (6) Discoloration of a piece of garlic or of a silver spoon in the pot is no indication of poisonous shellfish. The plain story of the chain of intoxication from the microscopic plant through the bivalve to man should be clearly understood by people along the coast, in order that they may avoid exposure to this type of food poisoning.

As for legislative control, various food and drug, fishery, and public health agencies in both Canada and the United States have taken measures for many years to prevent the occurrence of shellfish poisoning. Wherever poisonous clams are known to exist, digging is prohibited whenever tests demonstrate the meats to be toxic. In Alaska this may include nearly all seasons, because clams in certain localities remain toxic the year around. In California, mussels, being the most common source of the poison, are put under quarantine by the State Department of Public Health(1) from May 1 to November 1 and at such other times as laboratory tests show them to be dangerous.

During the last war, the commercial canning of clams in Alaska(7) grew rapidly from a negligible operation to one of significance to the territory. During the 1945-1946 season the operation was cut short by the finding of poison in packs of frozen clams. In January 1949 a tolerance level(7) was established for the amount of toxin in whole and minced clams. As modified(7) in February 1951, marketing of fresh, frozen or canned clams is permitted only when they have an average toxicity of less than 400 mouse units per 100 grams of contents.

No individual unit package is permitted to exceed 2,000 mouse units per 100 grams. In the case of minced or chopped clams, which are subject to less variation, the average toxicity must be less than 2,000 mouse units per 100 grams of contents and no unit package should materially exceed that figure.

The canning of clams in Canada around the Bay of Fundy and the mouth of the St. Lawrence River has also been practiced for many years. Almost 5,000 toxicity tests[32] have been made in this region since 1943, and they indicate that most of the growing areas in the Bay of Fundy can be readily classified for control purposes. Class I areas are not affected by paralytic shellfish poison at all. Class II areas are affected only in late summer for a short period each year, if at all. Class III areas are usually affected the year around. Furthermore, there is a consistent order in the time of appearance of the poison in different areas. Mussels in the areas exposed to the open Bay of Fundy regularly show poison about 10 days before the first traces appear in the tributary inlets. Accordingly, regular sampling at a "key station" shows when the time has come to impose the quarantine.

Up to and including 1945[9], canning of soft-shell clams was permitted in Canada at all seasons, but when toxicities of the raw stock were above 400 mouse units per 100 grams the pack was released for sale only after systematic sampling showed that it contained not more than 200 units per 100 grams of canned meat. Since processing normally destroys about 70 percent of the poison, such a product was regularly obtained[9] from raw shellfish containing 1000 mouse units or less per 100 grams. After the U. S. authorities relaxed their restrictions in 1951 on packs of whole Alaskan butter clams, the

Canadians[32] again adopted the 400 mouse unit quarantine level and now permit the marketing of clams (fresh, frozen or canned) when the average score of representative samples from each shipment is less than 400 and the scores of all samples are less than 2,000 mouse units per 100 grams.

Commercial harvesting of mussels[9] of any species from the Bay of Fundy, either for canning or for sale as raw food, has been prohibited at all seasons since the autumn of 1943, when their toxicities were found to be several times higher than those for clams. Likewise the marking of beaches or quarantine of an area to prevent local residents and tourists from digging clams or mussels has been generally instigated when the toxicity of the shellfish has been shown by laboratory tests to exceed 400 mouse units per 100 grams of meat.

These restrictions have interferred seriously with the shellfish industry[9] in Nova Scotia, New Brunswick, and Alaska. Nevertheless, they have proved necessary and do provide significant protection, as shown by the fact that sufficient poison[9] (1,000 to 5,000 units) to produce the mildest symptoms in the most susceptible persons would require an individual to eat a whole can, about 300 ml of meat and bouillon combined, with a toxicity of 300 to 1700 units per 100 ml.

For control purposes, the present accepted technique for determining the toxicity of shellfish is a bioassay using mice. Briefly, this method[9] involves the following steps. The clams are shucked, washed in fresh water, drained on a sieve for 5 minutes and minced in a meat grinder. A 100 gram portion of the ground meat is suspended in 100 ml of 0.1 N hydrochloric acid and boiled gently for 5 minutes with continuous stirring. The mixture is cooled, adjusted

to pH 4.0 or 4.5 by the addition of a few drops of 5 N acid or 0.1 N sodium hydroxide, and then made up to 200 ml. with distilled water. The supernatant liquid is clarified by settling or centrifuging and used for injection into a few test mice in order to obtain a preliminary estimate of potency. With high toxicity clams, dilutions of the extract may be necessary in order to obtain solutions which give death times within the desired range. The death times corresponding to various numbers of mouse units may be determined by referring to Sommer's Table*. When an injection of 1 ml. into each of three mice produces a mean death time of 10 to 20 minutes, each ml. is said to contain 1 mouse unit. The toxicity per 100 grams of meat is the number of mouse units contained in 1 ml. of extract multiplied by 200, which is the total volume of 100 grams of meat plus 100 ml. of dilute acid.

In 1949 the Fishery Products Research Laboratory sponsored a collaborative study[32] on the assay of toxic clam extracts. This study indicated that there is considerable variation when several strains of mice are used, and that the results from one laboratory may differ from the assays of another by as much as 60 to 70 percent under these circumstances.

In 1950 some changes in the assay procedure[33] were suggested, and further progress was made in 1955 when the Public Health Service sponsored a one-day conference[32] to discuss the most recent developments in the assay of shellfish poison. At the conference, the use

*Furnished by Sommer to other workers in this field. These tables are based on graphs[3] recorded in the literature. The essential portions have been reproduced for distribution by the Public Health Service[37].

of a purified shellfish poison as a reference standard was proposed by Dr. E. J. Schantz. Dr. Schantz and his associates at Fort Detrick have been engaged in this field of research for several years and have succeeded in isolating[34] the poison in pure form[35]. On the basis of the information presented at the conference, the conferees agreed (1) that the purified poison should be used as a reference standard for obtaining uniform bioassays, and (2) that the results of future bioassays should be reported in terms of the weight of the poison. As a result of the conference, the Robert A. Taft Sanitary Engineering Center cooperated with Dr. Schantz in determining practical requirements for the use of the purified poison as a reference standard. Briefly, it was found[36] that (1) a median death time between 5 and 7 minutes gives the most reliable estimate of potency; (2) in using the reference standard to determine the factor for conversion of mouse units to ug. of poison (CF value), the results should not vary by more than ± 20 percent; (3) the CF value obtained in one laboratory may differ significantly from that obtained in another laboratory through the use of different strains of mice or variations in technique of assay, but once the CF value has been determined in each laboratory it is expected that comparable results will be obtained in separate laboratories assaying clams, if the CF values are used to calculate the micrograms of poison; (4) in assaying low-toxicity clams containing around 400 mouse units per 100 grams, which is the present level of acceptability, the bioassay procedure may underestimate the total toxicity by as much as 60 percent.

The Public Health Service has recently issued an interim plan[37] for standardization of the bioassay and has secured a quantity of the

purified poison from the Chemical Corps for use as a reference standard. These items are now ready for distribution, and individuals or organizations desiring to obtain them should address their requests to The Department of Health, Education, and Welfare, Public Health Service, Washington 25, D. C., attention, Shellfish Sanitation Section.

As the result of the determination of the ug. of poison equivalent to one mouse unit, and the information, also presented at the conference in May 1955, that the poison gives a color reaction[28] in the Jaffe Test, a chemical method for the quantitative determination[39] of the poison has been devised. Briefly, this method (Figure II) consists of making an acid extract of the clam meats, adsorption of the extract on an ion-exchange resin, elution of the poison and measurement of it colorimetrically by the Jaffe Test.

FIGURE II

Flow Sheet for Chemical Assay of
Paralytic Shellfish Poison

↓

Extraction of 100 grams of Clam Meat with
100 ml. of 0.5 N Trichloroacetic Acid

↓

Adjust 100 ml. of Filtrate to pH 5.0

↓

Adsorb on XE-64 at pH 5.0

↓

Wash Column with 200 ml. of
pH 5.0 Buffer and 200 m. of
Water

↓

Elute with 0.5 N
Trichloroacetic Acid

↓

Apply Jaffe Color Test to Eluate

In Table IV are presented some data comparing the toxicities of various clam samples as determined by chemical and bioassay procedures. The data indicate that in testing low-toxicity clams the bioassay may underestimate the poison content by a considerable amount. This is as to be expected, since, as mentioned previously, in recovery studies in which poison was added to non-poisonous clams, the bioassay underestimated the toxicity by as much as 60 percent. In assaying clams of greater toxicity the bioassay becomes progressively better, until at high toxicities the recovery approaches 100%. In this case, as can be noted in Table IV, the bioassay agrees with the chemical assay. The chemical test may not be suitable for use in the field, but in the laboratory has accuracy equal to or greater than that of the bioassay, and should be useful as a control test particularly in areas where it is difficult to obtain and keep mice.

TABLE IV

Comparison of the Bioassay With the Chemical Assay for Determination of the Toxicity of Clams

Clam sample	Bioassay ug/100 g.	Chemical assay ug/100 g.
A	115	159
B	147	198
C	160	207
D	328	337
E	1093	1054

Another method which offers some promise of being useful as a field test is the chromate color test[40]. A brief description of this test is as follows: When an aqueous solution containing microgram quantities of shellfish poison and milligram quantities of chromium (VI) (H_2CrO_4) is made acidic, a blue color develops which is proportional to the amount of poison present. It has been possible to use a modification of this test in an assay of the acid extract of some species of clams.

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